

WHAT IS CLAIMED IS:

1. A method for producing sialyloligosaccharides in a dairy source comprising:

(i) contacting a catalytic amount of at least one $\alpha(2-3)$ trans-sialidase with a dairy source to form a dairy/trans-sialidase mixture; and

(ii) incubating said dairy/trans-sialidase mixture under conditions suitable for $\alpha(2-3)$ trans-sialidase activity.

2. The method of Claim 1 further comprising recovering sialyloligosaccharides from said incubated dairy/trans-sialidase mixture.

3. The method of Claim 1 further comprising the steps of:

(iii) processing said dairy/trans-sialidase mixture for cheesemaking to form a cheese processing waste stream; and

(iv) recovering sialyloligosaccharides from said cheese processing waste stream.

4. The method of Claim 1 wherein the $\alpha(2-3)$ trans-sialidase is a Kinetoplastid trans-sialidase.

5. The method of Claim 1 wherein the $\alpha(2-3)$ trans-sialidase is encoded by a gene isolated from a species of the genera

selected from the group consisting of *Trypanosoma*, *Endotrypanum* and *Pneumocystis*.

6. The method of Claim 1 wherein the $\alpha(2-3)$ trans-sialidase is recombinantly produced.

7. The method of Claim 1 wherein the dairy source comprises a member selected from the group consisting of milk, colostrum, and cheese processing mixture.

8. The method of Claim 1 wherein the dairy source/trans-sialidase mixture is incubated for at least 1 hour.

9. The method of Claim 1 wherein the dairy source/trans-sialidase mixture is incubated at a temperature of about 5°C to about 45°C.

5 10. The method of Claim 1 wherein the dairy source/trans-sialidase mixture has a pH of about 6 to about 8.

11. The method of Claim 3 wherein the cheese processing waste stream comprises a member selected from the group
10 consisting of: whole whey, demineralized whey permeate, a regeneration stream from demineralized whey permeate, whey permeate, crystallized lactose, spray dried lactose, whey powder, edible lactose and lactose.

15 12. The method of Claim 2 wherein the recovering step comprises ultrafiltration of the incubated dairy source/trans-sialidase mixture to form an ultrafiltrate.

13. The method of Claim 3 wherein the recovering step
20 comprises ultrafiltration of the cheese processing waste stream to form an ultrafiltrate.

14. The method of Claim 12 or 13 wherein the recovering step further comprises contacting said ultrafiltrate with an ion
25 exchange resin.

15. The method of Claim 14 wherein the ion exchange resin is an anion exchange resin.

30 16. The method of Claim 14 wherein the ion exchange resin is a cation exchange resin.

17. The method of Claim 2 wherein the recovering step comprises:

35 (a) contacting said incubated dairy source/trans-sialidase mixture of step (ii) with a solvent and extracting

sialyloligosaccharides with said solvent to form a sialyloligosaccharide-containing solvent;

(b) separating said sialyloligosaccharide-containing solvent from said incubated dairy source/*trans*-sialidase mixture; and

(c) isolating said sialyloligosaccharides from said sialyloligosaccharide-containing solvent.

18. The method of Claim 3 wherein the recovering step comprises:

(a) contacting said cheese processing waste stream with a solvent and extracting sialyloligosaccharides with said solvent to form a sialyloligosaccharide-containing solvent;

(b) separating said sialyloligosaccharide-containing solvent from said cheese processing waste stream; and

(c) isolating said sialyloligosaccharides from said sialyloligosaccharide-containing solvent.

19. A method for producing sialyloligosaccharides in a cheese processing waste stream comprising:

(i) contacting a catalytic amount of at least one $\alpha(2-3)$ *trans*-sialidase with a cheese processing waste stream to form a waste stream/*trans*-sialidase mixture; and

(ii) incubating said waste stream/*trans*-sialidase mixture under conditions suitable for $\alpha(2-3)$ *trans*-sialidase activity.

20. The method of Claim 19 further comprising recovering sialyloligosaccharides from said incubated waste stream/*trans*-sialidase mixture.

21. The method of Claim 19 wherein said $\alpha(2-3)$ *trans*-sialidase is a Kinetoplastid *trans*-sialidase.

22. The method of Claim 19 wherein said $\alpha(2-3)$ *trans*-sialidase is encoded by a gene isolated from a species of the genus *Trypanosoma*, *Endotrypanum*, or *Pneumocystis*.

23. The method of Claim 19 wherein said $\alpha(2-3)$ trans-sialidase is recombinantly produced.

24. The method of Claim 19 wherein the waste stream/trans-
5 sialidase mixture is incubated for at least 1 hour.

25. The method of Claim 19 wherein the waste stream/trans-sialidase mixture is incubated at a temperature of about 5°C to about 45°C.

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26. The method of Claim 19 wherein the waste stream/trans-sialidase mixture has a pH of about 5 to about 8.

27. The method of Claim 19 wherein the cheese processing
15 waste stream comprises a member selected from the group consisting of: whole whey, demineralized whey permeate, the regeneration stream from demineralized whey permeate, whey permeate, and whey powder.

20 28. The method of Claim 20 wherein the recovering step comprises ultrafiltration of the incubated waste stream/trans-sialidase mixture to form an ultrafiltrate.

29. The method of Claim 28 wherein the recovering step
25 further comprises contacting said ultrafiltrate with an ion exchange resin.

30. The method of Claim 29 wherein the ion exchange resin is an anion exchange resin.

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31. The method of Claim 29 wherein the ion exchange resin is a cation exchange resin.

32. The method of Claim 20 wherein the recovering step
35 comprises:

(a) contacting said incubated waste stream/trans-sialidase mixture of step (ii) with a solvent and extracting

said sialyloligosaccharides with said solvent to form a sialyloligosaccharide-containing solvent;

(b) separating said sialyloligosaccharide-containing solvent from said incubated waste stream/trans-sialidase mixture; and

(c) isolating said sialyloligosaccharides from said sialyloligosaccharide-containing solvent.

33. The method of Claim or 17, 18 or 32 wherein said solvent is selected from the group consisting of water, C[1-5] alcohol and a mixture thereof.

34. The method of Claim 3 or 19 wherein said cheese processing waste stream is the mother liquor obtained by crystallizing lactose from cheese whey.

35. The method of Claim 1 or 19 wherein exogenous $\alpha(2-3)$ sialyloligosaccharides are added during said incubating step.

36. A method for producing $\alpha(2-3)$ sialyllactose comprising:
(i) contacting a catalytic amount of at least one $\alpha(2-3)$ trans-sialidase with lactose and an $\alpha(2-3)$ sialyloligosaccharide, in the absence of CMP-sialyltransferase, to form a mixture; and
(ii) incubating said mixture under conditions suitable for $\alpha(2-3)$ trans-sialidase activity.

37. A transgenic mammal comprising an $\alpha(2-3)$ trans-sialidase encoding sequence operably linked to a regulatory sequence of a gene expressed in mammary tissue.

38. The transgenic mammal of Claim 37 wherein said regulatory sequence is derived from a gene encoding a milk specific protein.

39. The method of Claim 38 in wherein said regulatory sequence is derived from a gene encoding a protein selected

from the group consisting of: whey acidic protein, β -lactoglobulin, α -lactalbumin, α s1-casein and β -casein.

40. The transgenic mammal of Claim 37 wherein the $\alpha(2-3)$
 5 *trans*-sialidase encoding sequence encodes a kinetoplastid *trans*-sialidase.

41. The transgenic mammal of Claim 37 wherein said $\alpha(2-3)$
 trans-sialidase encoding sequence hybridizes under high
 10 stringency conditions to an $\alpha(2-3)$ *trans*-sialidase gene
 selected from the group consisting of *Trypanosoma cruzi*, *Trypanosoma brucei*, *Endotrypanum spp.* and *Pneumocystis carinii*.

15 42. The transgenic mammal of Claim 37 in which the
 transgenic mammal is a cow, sheep, pig or goat.

43. A method for enriching for $\alpha(2-3)$ sialyllactose in milk
 comprising:

- 20 (i) introducing a transgene comprising an $\alpha(2-3)$ *trans*-
sialidase encoding sequence operably linked to a regulatory
sequence of a gene expressed in mammary tissue into the
germline of a mammal to produce a transgenic mammal;
 (ii) selecting a transgenic mammal demonstrating $\alpha(2-3)$
 25 *trans*-sialidase activity; and
 (iii) obtaining milk from the selected transgenic
mammal.

44. The method of Claim 43 further comprising recovering
 30 $\alpha(2-3)$ sialyllactose from said milk.

45. An $\alpha(2-3)$ sialyllactose formed by the process comprising
 contacting a catalytic amount of at least one $\alpha(2-3)$ *trans*-
sialidase with a dairy source to form a dairy/*trans*-sialidase
 35 mixture; and incubating said dairy/*trans*-sialidase mixture
 under conditions suitable for $\alpha(2-3)$ *trans*-sialidase
 activity.

46. An $\alpha(2-3)$ sialyllactose formed by the process comprising contacting a catalytic amount of at least one $\alpha(2-3)$ *trans*-sialidase with a cheese processing waste stream to form a waste stream/*trans*-sialidase mixture; and incubating said
5 waste stream/*trans*-sialidase mixture under conditions suitable for $\alpha(2-3)$ *trans*-sialidase activity.

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